In the Specification:

Please replace the invention title at page 1, lines 1-2 with the following substitute title:

~~NOVEL GENE ENCODING A DNA REPAIR ENZYME AND METHODS OF USE THEREOF~~ ANTIBODIES IMMUNOLOGICALLY SPECIFIC FOR A DNA REPAIR ENDONUCLEASE AND METHODS OF USE THEREOF

Please replace the paragraph at page 1, lines 5-15 with the following substitute paragraph:

This application is a continuation of US Application 09/629,222, filed July 31, 2000, which is a continuation-in-part application of US Application 09/463,891 ~~entitled "Novel Gene Encoding a DNA Repair Endonuclease and Methods of Use Thereof"~~, filed January 28, 2000, now abandoned, which is the US National Phase Application of PCT/US98/15828, filed July 28, 199~~9~~8. This application also claims priority under 35 U.S.C. §119(e) from US Provisional Application 60/053,936 filed July 28, 1997. The entire disclosure of all of the above-identified applications are incorporated by reference herein.

Please replace the paragraph begins at page 13, line 35, ends at page 14, line 8 of the specification with the following substitute paragraph:

~~Figure 4A depicts homology analysis of the deduced amino acid sequence of MED1 and several other endonucleases involved in DNA recognition and~~

~~repair.~~ Figure 4~~B~~A depicts homology analysis of the deduced amino acid sequence of MED1 (SEQ ID NO: 30) and the methyl-CpG binding domain of the rat protein, MeCP2 (SEQ ID NO: 31). Figure 4B depicts homology analysis of the deduced amino acid sequence of MED1 (line a, SEQ ID NO: 32) and several other endonucleases involved in DNA recognition and repair (line b, SEQ ID NO: 33; line c, SEQ ID NO: 34; line d, SEQ ID NO: 35; line e, SEQ ID NO: 36). Figure 4C depicts homology analysis of the deduced amino acid sequence of MED1 (SEQ ID NO: 37) and the methyl-CpG binding domain of the human protein, PCM1 (SEQ ID NO: 38).

Please replace the paragraph at page 17, lines 6-20 of the specification with the following substitute paragraph:

Figures 12A and 12B show~~s~~ a series of MED1 mutations which have been isolated from colon cancer patients. Figures 12A and 12B ~~and~~ show MED1 sequencing electropherograms (ABI) of three colon tumor DNAs and a normal control DNA (SEQ ID NO: 39 in Fig. 12A and SEQ ID NO: 41 in Fig. 12B). Tumors c220T (top sequence: SEQ ID NO: 39; bottom sequence: SEQ ID NO: 40) and c226T (top sequence: SEQ ID NO: 39; bottom sequence: SEQ ID NO: 40) harbor an apparently heterozygous adenine deletion at the (A)10 track (codons 310-313) with predicted frameshift and stop at codon 317 (Fig. 12A). The same mutation was also found in tumor c18T. Tumor c215T (top sequence: SEQ ID NO: 41; bottom sequence: SEQ ID NO: 42) harbors an apparently heterozygous adenine deletion at the (A)6 track (codons 280-282) with predicted frameshift and stop at codon 302

(Fig. 12B). Figure 12C shows a schematic diagram of the truncated products predicted to be encoded by the mutant MED1 alleles in the indicated tumors.

Please replace the paragraph at page 18, lines 19-22 of the specification with the following substitute paragraph:

Figures 16A and 16B shows the nucleotide sequence (SEQ ID NO: 5) of the mouse cDNA MED1 sequence assembled by juxtaposition of seven exons derived from the genomic clone MED1 MGL #3. Amino Acid Sequence= SEQ ID NO:29.

Please replace the paragraph at Page 18, lines 24-30 of the specification with the following substitute paragraph:

Figure 17 shows a comparison of the predicted mouse MED1 protein sequence with the human MED1 protein sequence. Upper sequence: mouse MED1 (SEQ ID NO: 29); lower sequence: human MED1 (residues 36 to 285 and residues 386 to 555 of SEQ ID NO: 2). Identical amino acids between the two sequences are indicated by a line, similar amino acids by one (low similarity) or two dots (high similarity).

Please replace the paragraph begins at page 18, lines 32, ends at page 19, line 1 of the specification with the following substitute paragraph:

Figures 18A (SEQ ID NO: 21), 18B (SEQ ID NO: 44), 18C (SEQ ID NO: 45), 18D (SEQ ID NO: 46), 18E (SEQ ID NO: 47), and 18F (SEQ ID NO: 48) shows the intron and exon sequences of the mouse genomic clone encoding MED1. Exon sequences are shown in upper

case; intron sequences are shown in lower case. The splice donor (gt) and acceptor (ga) sites are in bold.

Please replace the paragraph at page 19, lines 3-7 of the specification with the following substitute paragraph:

Figures 19A (SEQ ID NO: 22), 19B (SEQ ID NO: 49), 19C (SEQ ID NO: 50), 19D (SEQ ID NO: 51), 19E (SEQ ID NO: 52), 19F (SEQ ID NO: 53), 19G (SEQ ID NO: 54), and 19H (SEQ ID NO: 28) shows the intron and exon sequences of the human genomic clone encoding MED1. Exon sequences are shown in upper case; intron sequences are shown in lower case. The splice donor (gt) and acceptor (ga) sites are in bold.

Please replace the paragraph at page 59, lines 1-23 of the specification with the following substitute paragraph:

D. Cell culture, expression constructs, and transfections.

NIH 3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, penicillin (50 units/ml), streptomycin (50µg/ml), and kanamycin (100 µg/ml). The expression constructs of MED1 (SEQ ID NO: 1) were generated in the CMV promoter-based CMV5 vector, a derivative of CMV4 (Andersson et al., (1989) *J. Biol. Chem.* 264:8222-8229). For construction of the hemagglutinin epitope carboxy-terminally tagged MED1 plasmid, the MED1 cDNA was inserted in place of the Gfi-1 ZN mutant construct open reading frame (Grimes et al. (1996) *Mol. Cell Bio.* 16:6263-6272), a gift

of Dr. Leighton Grimes. For construction of the hemagglutinin epitope amino terminally-tagged MED1 plasmids M1 and M2, a XbaI site was inserted by polymerase chain reaction immediately upstream of the ATG codons at nucleotide positions 142 and 262, respectively. Then the MED1 open reading frame, excised with XbaI and NsiI (blunted), was inserted in place of the Akt gene in the CMV5 hemagglutinin tag-Akt construct (Datta et al., (1996) *J. Biol. Chem.* 271:30835-30839).

Please replace the paragraph begins at page 65, line 24, ends at page 66, line 12 of the specification with the following substitute paragraph:

K. Endonuclease activity of recombinant wild-type MED1.

The entire wild-type MED1 (amino acids ~~codons~~ 1-580, wt (SEQ ID NO: 2)) and a deletion mutant lacking the endonuclease domain (amino acids ~~codons~~ 1-454, Δendo) were expressed in bacteria and purified by nickel-agarose chromatography. For bacterial expression, PCR-generated fragments corresponding to the entire MED1 open reading frame or to isolated domains were propagated in *E. coli* strain XL-1 Blue (Stratagene) and transferred into pET28(b) (Novagen). Constructs were sequenced with an automated DNA sequencer (ABI) to verify that unwanted mutations were not inadvertently introduced; and they were transferred into *E. coli* strain BL21(DE3)pLysS. These cells were grown to O.D.600= 0.4 and then induced with 1 mM IPTG at 37°C for 3 hours. Bacterial lysates were purified over a nickel-agarose column (Ni^{2}+-NTA agarose, Qiagen).

Increasing amounts of the wild-type and Δendo mutant (22, 44, 87.5 and 175 ng) were incubated with 500 ng of the 3.9 kb supercoiled plasmid pCR2 (Invitrogen) at 37°C for 30 min in a buffer containing 20 mM Tris-HCl pH 7.5, 25 mM KCl and 10 mM $MgCl_2$. Reaction products were separated on a 1% agarose gel buffered in 1x TAE and containing 0.25 µg/ml ethidium bromide.

Please replace the paragraph begins at page 68, line 29, ends at page 69, line 3 of the specification with the following substitute paragraph:

1) Nucleotides 1325-1342: 18 nucleotides - GTGAGAAAATATTTCAAG (nucleotides 1325 through 1342 of SEQ ID NO: 1) - are either present (as in Sequence I.D. No. 1) or absent (as in Sequence I. D. No. 23) from the cDNA, therefore the 6 amino acids encoded by those nucleotides (GEKIFQ; residues 395 through 400 of SEQ ID NO: 2) are either present (as in Sequence I. D. No. 2) or absent (as in Sequence I. D. No.24) in the predicted protein. This variation appears to originate from alternative usage of a splice donor site. In the genomic DNA sequence:

...GACTTCACTGGTGAGAAAATATTTCAAG**GT**.. (SEQ ID NO: 73)

Please replace the paragraph at page 69, lines 4-9 of the specification with the following substitute paragraph:

If the second splice donor site (bold) is used, then the 18 nucleotides GTGAGAAAATATTTCAAG (nucleotides 1325 through 1342 of SEQ ID NO: 1) are incorporated in the mRNA; if the first splice donor

site (underlined) is used, then the same 18 nucleotides are spliced out and are not incorporated in the mRNA.

Please replace the paragraph begins at page 70, line 25, ends at page 71, line 28 of the specification with the following substitute paragraph:

In order to confirm that the MED1 open reading frame (SEQ ID NO: 1) is capable of directing the synthesis of a protein product, a construct of MED1 (SEQ ID NO: 1) in the vector pcDNA3 was employed in an *in vitro* coupled transcription and translation assay. The result indicated that the MED1 open reading frame (SEQ ID NO: 1) drives the translation of two polypeptides of 70 and 65 kD, shown in Figure 6, in good agreement with the molecular weight predicted from the amino acid sequence. The synthesis of these two polypeptides might be the result of initiation from the two close ATG codons, at nucleotide positions 142 and 262, respectively. Such a possibility is known to occur as a result of "leaky" ribosome scanning and is increased by a suboptimal Kozak's context (Kozak, M., (1995) *Proc. Natl. Acad. Sci.* 92:2662-2666). The difference in molecular weight (5kD) would be compatible with the distance between the two ATG codons (40 a.a.). To determine if two MED1 proteins are also synthesized *in vivo*, a hemagglutinin epitope was fused at the carboxyterminal end of the MED1 open reading frame (SEQ ID NO: 1), generating the construct MED1-HT. Constructs were also generated which fused a hemagglutinin tag immediately before each of the two putative initiation codons (HT-MED1-M1 and

HT-MED1-M2). These constructs were transiently transfected in NIH-3T3 cells and lysates of the transfectants were prepared and separated by SDS-PAGE. Western analysis with an anti-hemagglutinin tag antibody revealed the presence of a band of approximately 72 kD in cells transfected with the carboxyterminally tagged MED1-HT. This band comigrates with the one present in HT-MED1-M1 transfectants, indicating that the first ATG at nucleotide position 142 is the initiation codon *in vivo*. See Figure 7. Since the expression level of HT-MED1-M1 which uses the hemagglutinin tag ATG codon is much higher than MED1-HT which uses the autologous ATG codon, it is possible that the expression of the MED1 protein is under a tight translational control.

Please replace the paragraph begins at page 72, line 16, ends at page 73, line 5 of the specification with the following substitute paragraph:

To better define its nuclease properties, the entire MED1 protein (SEQ ID NO: 2) was expressed in *E. coli* as a carboxyterminal fusion to a six-histidine tag and purified on a nickel-agarose column to approximately 95% homogeneity. See Figure 9B, left panel. Endonuclease activity was assayed by evaluating the conversion of a supercoiled plasmid into open circles (nicked) and linear molecules. Increasing amounts of the purified MED1 protein were incubated with supercoiled plasmid DNA at 37°C for 30 min, and the products of the reactions, separated on a 1% agarose gel, were visualized by ethidium bromide staining. Incubation

with MED1 resulted in a dose-dependent appearance of nicked and linearized molecules (Fig. 9B, right panel). In order to rule out the possibility that a bacterial endonuclease activity copurifying with MED1 is responsible for the observed effects, a deletion mutant lacking the putative endonuclease domain was also purified. This mutant failed to produce nicked and linearized DNA molecules (Fig. 9B, right panel). These results indicate that MED1 has single- and double-strand endonuclease activity on a supercoiled plasmid substrate. Additional studies of the catalytic activity of MED1 are presented in Example V.

Please replace the paragraph begins at page 73, line 18, ends at page 74, line 7 of the specification with the following substitute paragraph:

To assess whether the MED1 methyl-CpG binding domain (MBD) is able to bind methylated DNA, a FLAG epitope was fused at the amino terminal end of the MED1 open reading frame (SEQ ID NO: 1), generating the construct FT-MED1/f5, and this construct was transfected into the human kidney line 293. Cells were also transfected with the empty expression vector. Seventy-two hours after transfection, cells were lysed and the lysates were immunoprecipitated with an anti-Flag antibody coupled to agarose beads. Bound protein was eluted from the beads following incubation with a FLAG peptide. The FT-MED1/f5 and control eluates were incubated with a ^{32}P-labeled double-stranded oligonucleotide containing a total of five fully methylated CpG sites, in the presence or absence of a 100-fold excess of the unlabeled or

"cold" oligonucleotide. The binding reactions were separated on a non-denaturing polyacrylamide gel and detected by autoradiography of the dried gel. A slowly migrating band was detected in the FT-MED1/f5 eluate lanes, but not in the control lane. This band was abolished by competition with excess cold oligonucleotide. This experiment indicated that the MBD of MED1 functions as a specific methylated DNA binding domain in vivo. See Figure 10A.

Please replace the paragraph at page 74, lines 8-31 of the specification with the following substitute paragraph:

To further characterize the DNA binding properties of MED1 (SEQ ID NO: 2), its putative methyl-CpG binding domain (MBD) was expressed in *E. coli* as a carboxyterminal fusion to a six-histidine tag, and it was purified by metal-chelating affinity chromatography followed by ion-exchange chromatography on SP Sepharose (Pharmacia). The purity of the MED1 MBD was estimated at >98% by SDS-PAGE followed by Coomassie staining. The purified MBD was incubated with a ^{32}P-labeled double-strand oligonucleotide of arbitrary sequence containing five symmetrical methyl-CpG sites. As a control, MBD was incubated with a ^{32}P-labeled double-strand oligonucleotide of identical sequence in which cytosines replaced 5-methyl-cytosines. EMSA analysis of the complexes indicated that the MED1 MBD binds to methylated DNA and fails to bind to unmethylated DNA (Fig. 10B, lanes 2 and 6). Binding to the methylated probe was competed by preincubation with a 100-fold excess of cold methylated oligonucleotide (lane 3). Little

competition was observed following preincubation with the unmethylated oligonucleotide (Fig. 10B, lane 4). This experiment provides further evidence of the methyl-CpG binding specificity of the MED1 MBD.

Please replace the paragraph at page 79, lines 23-32 of the specification with the following substitute paragraph:

A common feature of the MED1-related endonucleases is the presence of a Cys-X6-Cys-X2-Cys-X5-Cys sequence (SEQ ID NO: 43) at their carboxy terminus. This sequence, as shown in endonuclease III, ligates the [4Fe-4S] iron-sulfur cluster and defines a novel DNA binding motif (named the FCL motif), which provides the correct alignment of the enzyme along the DNA (Thayer et al., (1995) Embo J. 14:4108-4120). MED1 lacks a FCL motif at its carboxy terminus, but contains a methyl-CpG DNA binding domain at the amino terminus.

Please replace the paragraph at page 81, lines 9-18 of the specification with the following substitute paragraph:

SSCP analysis was performed by PCR amplification of two MED1 segments encompassing the coding microsatellite repeat regions poly$(A)_{10}$ and poly$(A)_6$. In particular, PCR reactions were carried out with the MED1 primers 5'-CTCGTTGTGTTCTGAGCTTTTGGC-3' (SEQ ID NO: ~~30~~55) and 5'-CAGTGTGACCAGTGAAGAAAA-3' (SEQ ID NO: ~~31~~56) for analysis of the $(A)_{10}$ repeat at codons 310-313; and 5'-TGAAAGGAATCCCAATTAAG-3' (SEQ ID NO: ~~32~~57) and 5'-

GACAGTTCTATCAAGCTGAC-3' (SEQ ID NO: ~~33~~58) for analysis of the $(A)_6$ repeat at codons 247-248.

Please replace the paragraph begins at page 87, line 26, ends at page 88, line 4 of the specification with the following substitute paragraph:

5' CCGTCATGCTAGTTCACTTTATGCTTCCGGCTC**X**CGTCATGTGTGGAATTGTGATTAAAATCG
3' (SEQ ID NO: 59)
3' GCAGTACGATCAAGTGAAATACGAAGGCCGAG**Y**GCAGTACACACCTTAACACTAATTTTAGCG
5' (SEQ ID NO: 60)
X= A,G,C,T
Y= A,G,C,T,U,E

5' CCGTCATGCTAGTTCACTTTATGCTTCCGGCT**ZG**CGTCATGTGTGGAATTGTGATTAAAATCG
3' (SEQ ID NO: 61)
3' GCAGTACGATCAAGTGAAATACGAAGGCCGA**WJ**GCAGTACACACCTTAACACTAATTTTAGCG
5' (SEQ ID NO: 62)
Z:W= A:T,G:C,C:G,T:A
J= T,U

K
5' CCGTCATGCTAGTTCACTTTATGCTTCCGGCTCG^CGTCATGTGTGGAATTGTGATTAAAATCG
3' (SEQ ID NO: 63)
3' GCAGTACGATCAAGTGAAATACGAAGGCCGAGC.GCAGTACACACCTTAACACTAATTTTAGCG
5' (SEQ ID NO: 70)
K= Ins A,G,C,T,GT (SEQ ID NO: 64),GTA (SEQ ID NO: 65),GTAC (SEQ ID NO: 66), GTACT (SEQ ID NO: 67), GGGGG (SEQ ID NO: 68); del C (SEQ ID NO: 69)

5' CAATCCTAGCTGACAC**G**ATGTGGCCAATGGCATGACT 3' (SEQ ID NO: 71)
3' TTAGGATCGACTGTG**@**TACACCGGTTACCGTACTGAG 5' (SEQ ID NO: 72)

@= C,T,U,E
G= Guanine, C= Cytosine, T= Thymine, A= Adenine, U= Uracyl, E= etheno Cytosine